Epibiont populations associated with *Diporeia* spp. (amphipoda) from Lake Michigan

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Introduction

The benthic amphipod *Diporeia* spp. is the dominant benthic macroinvertebrate in the offshore region (>30 m) of the Laurentian Great Lakes and, as a detritivore, plays a critical role in the offshore food web. *Diporeia* feed on organic material that settles from the water column and, in turn, are fed upon by many fish species. This organism, therefore, serves as an important trophic link between lower and upper trophic levels (GARDNER et al. 1990). *Diporeia* are currently declining in all the Great Lakes except Lake Superior (DERMOTT & KEREC 1997, NALEPA et al. 1998, LOZANO et al. 2001, DERMOTT 2001). While declines have been coincidental with the introduction and spread of the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena bugensis*), exact mechanisms for the negative response have not been clearly defined (NALEPA et al. 2004).

To examine the hypothesis that epibiont protozoans may be causing the loss of *Diporeia*, we documented the quantity, kind and distribution pattern of epibionts attached to *Diporeia* at three sites in Lake Michigan where populations were in various states of decline. A recent histological survey of *Diporeia* from Lakes Michigan and Huron identified various potential pathogens associated with *Diporeia* including epibionts, microsporidians, haplosporidians and fungi (MESSICK et al. 2004). This same histological survey found that 37% of all collected individuals were infested with epibiont protozoans, but the quantity, kind and distribution pattern of the attached epibiont forms were not documented. Extensive epibiont colonization, particularly on sensitive areas such as the gills, may have a negative long-term effect on crustacean populations (VOGELBEIN & THUNE 1988, COOK et al. 1998). We employed scanning electron microscopy (SEM) to examine *Diporeia* and record attached epibionts (WALKER & ROBERTS 1982, COOK et al. 1998, ROBERTS & CHUBB 1998). While the term "epibiotic" can be used to describe the relationship between a variety of microorganisms (i.e. algae, protozoans, bacteria, and fungi) and their host (CARMEN & DOBBS 1997), in this paper we use the term to describe primarily the relationship between protozoa and *Diporeia*.

Key words: ciliates, amphipod, epibionts, freshwater, Lake Michigan

Materials and methods

Diporeia were collected on a monthly basis between April and November 2002 at three sites in southeastern Lake Michigan. Based on previous sampling efforts between 1998 and 2001, we knew that *Diporeia* populations were in different states of decline at these sites (NALEPA unpubl. data). *Diporeia* had declined severely at Station M-45 (45 m depth; 43 11.27 N, 86 25.78 W); mean densities were 5500 m^{-2} in 1998 but only 250 m^{-2} in 2001. Densities at the other two sites were more stable. Densities at Station L-2 (45 m depth; 43 30.05 N, 86 31.88 W) were 7300 m^{-2} and 5300 m^{-2} in 1998 and 2001, respectively, whereas densities at Station X-2 (100 m depth; 43 12.00 N and 86 31.00 W) were 5200 m^{-2} and 4000 m^{-2} in 1998 and 2001, respectively. On each sampling date, triplicate samples were taken with a Ponar grab (sampling area = 0.046 m^2), washed through an elutriation device fitted with a 0.5-mm mesh sleeve, and retained material was preserved in 5% calcium carbonate buffered formalin (NALEPA et al. 1998, 2000). Additional Ponar grabs were taken at each station on each date for the collection of live animals that were later examined for epibionts. These intact grabs were placed individually into plastic bags and kept cool during transport back to the laboratory.

In the lab, all *Diporeia* in the preserved samples were picked and counted under a 1.5 x magnification lamp. Live *Diporeia* from the intact samples were sorted into two size classes (<5 mm and >5 mm), and ten individuals from each size class were placed in 5% buffered formalin. After 24 h, animals were dehydrated in a graded series of ethanol concentrations (30%>50%>70%>90%>100%) for 10 min at each concentration. Following the ethanol changes, *Dipor-*

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eia were exposed to three changes of hexamethyldisilazane (30 min ea.). After the third change, the hexamethyldisilazane was allowed to completely evaporate before the animals were placed in 1% Osmium tetroxide (WALKER et al. 1996, NATION 1983). Animals were mounted ventral side up, on aluminum stubs, using carbon permeated double stick tape and colloidal graphite with pereiopods and pleopods spread adjacent to the body. Animals were then sputter-coated using a Technics model Hummer V sputter coater and examined using an Amray 1820 I SEM with an acceleration voltage of 5kV.

To document epibiont distribution patterns, we established four regions on *Diporeia* (I-Cephalic, II-Thorax, III-Mid-Body, and IV-Caudal) with regions divided into sub-regions when appropriate (i.e., antennae, head, mouthparts, body, pereiopods, gills, pleopods, oostegites, and telson). Therefore, during the enumerating process, the body location for each epibiont was noted and placed into both a major region as well as a subregion. Dorsal surfaces of 20 animals were also examined for epibionts. Since minimal numbers were found on the dorsal surface in this study as well as in other studies (COOK et al. 1998), only results for the ventral surface are presented here. Epibiont taxonomy classification was taken to family level whenever possible using CORLISS (1979).

Results

Over all dates and sites, epibionts were found on 170 of the 173 *Diporeia* examined, and the mean (± SD) number of epibionts per *Diporeia* was 104 ± 65 (Table 1), six taxonomic groups were identified: Epistylidae, Lagenophryidae, Stentoridae, Acinetidae, Unknown 1, and Unknown 2 (Fig. 1). Unknown 1 was ovate shaped, measuring about 20 µm long and 12–15 µm wide, and had no distinguishing external characteristics. Unknown 2 measured about 10 µm long by 10 µm wide, was nearly spherical in appearance, and had many projecting rods measuring about 0.5 µm wide by 3 µm long. The most common epibiont was the peritrich Epistylidae, accounting for 77.5% of all epibionts found. It was present on 97% of all *Diporeia* and had a mean abundance of 81 ± 46 per individual. The next most common taxa was Unknown 1; it accounted for 11.5% of all epibionts, was found on 85% of all *Diporeia*, and had a mean abundance of 12 ± 11 per individual. All other taxa were found on <50% of *Diporeia* and had abundances of <7 per individual (Table 1).

During the sampling period, relative densities of *Diporeia* at M-45 and X-2 remained consistently low and high, respectively. Mean densities at the two sites were 351 m^{-2} and 4010 m^{-2} (Fig. 2). In contrast, densities at L-2 declined from a mean of 4040 m^{-2} in April/May to 250 m^{-2} in November, a 94% decline in just six months. Differences in numbers of epibionts at the three stations were examined using two-way ANOVA (station x season). There was no significant difference in the total number of epibionts found attached to *Diporeia* at the three sites over all sampling dates ($F = 1.84$, $P = 0.16$). When each of the six identified groups was examined separately, however, there was a significantly greater number of Epistylidae at M-45 ($F = 4.06$, $P = 0.02$; Tukey's LSD). The station x season interaction was also significant ($F = 2.89$, $P = 0.02$). The number of attached Epistylidae at M-45 increased from spring to fall. The number found at M-45 in the fall was more than twice the number found at X-2 (Fig. 3); mean (± SE) numbers found in the fall were 122 ± 16, 82 ± 23, and 52 ± 7 per individual at M-45, L-2, and X-2, respectively. The number of attached epibionts also increased at L-2 in the fall, but the increase was only observed in November.

The distribution pattern of epibionts attached to the body was similar at the three sites (Fig. 4). The greatest numbers of epibionts (67%) were found on the anterior portion of *Diporeia* attached to the pereiopods and body surface in the Thorax (II) and Mid-body (III) regions. Although only 5% of all epibionts were found on the coxal gills, the quantity and kind of epibionts found on the gills were of special interest because of implications to respiratory capacity and long-term stress. Of the six recognized taxa, the only one found on the gills was the peritrich Lagenophryidae. The mean number of *Lagenophyrs* spp. per *Diporeia* was three (maximum 70) and, as noted, no significant difference in the number was found at the three sites (ANOVA; $P = 0.34$).

Discussion

Diporeia populations in all the Great Lakes except Lake Superior have declined since the invasion of *Dreissena* (DERMOTT & KEREC 1997,

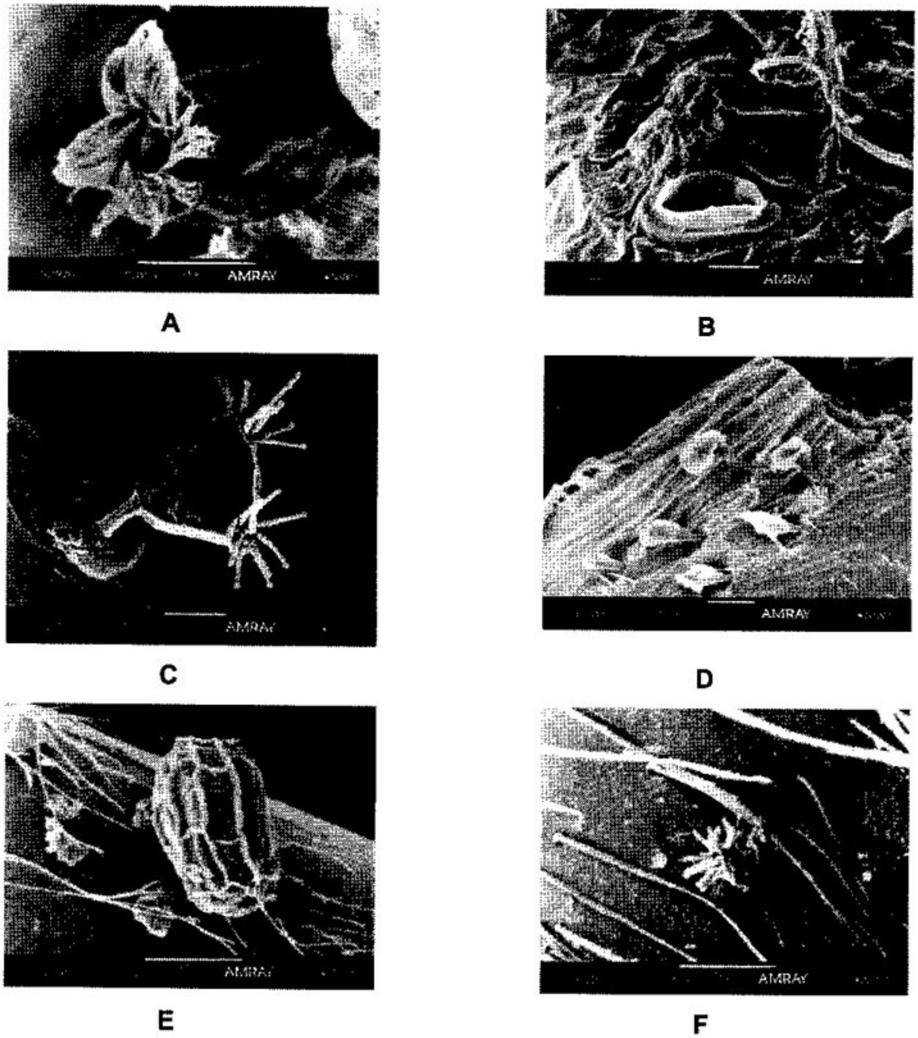

Fig. 1. The six major epibiont groups observed on *Diporeia*. **A)** Epistylidae, **B)** Lagenophryidae, **C)** Acinetidae, **D)** Stentoridae, **E)** Unknown 1, **F)** Unknown 2

NALEPA et al. 1998, 2003, LOZANO et al. 2001, DERMOTT 2001). We explored the hypothesis that changes in epibiont loads of *Diporeia* may be a factor in these declines. *Dreissena* may have enhanced epibiont populations either directly or indirectly through their metabolic activities (i.e. biodeposition, nutrient excretion) as was observed with meiofauna in Lake Erie (DERMOTT & KEREC 1997). Enhanced epibiont populations may increase burdens associated with *Diporeia*. Also, LOWE & PILLSBURY (1995) found a shift in benthic algal community structure and function following *Dreissena* establishment in Saginaw Bay, Lake Huron and, potentially, similar changes in benthic microbial (i.e. bacteria and fungi) and protozoan communities may have occurred. LAVRENTYEV et al. (2000) found that filter-feeding bivalves could significantly affect nitrogen transformation rates near the sediment-water surface, altering the microbial food web at the sediment-water surface. Another possibility is that a new

Table 1. The prevalence, mean intensity, and mean (± SD) abundance of major epibiont groups found attached to *Diporeia* at three sites in Lake Michigan in 2002. Prevalence (%) = (number of *Diporeia* with an epibiont group) / (total number of *Diporeia* examined in sample); Mean Intensity = (total number of an epibiont group) / (total *Diporeia* infected with that epibiont group); Mean Abundance = (total number of an epibiont group) / (total *Diporeia* examined in sample).

Station	Epibiont	Prevalence (%)	Mean Intensity	Mean (±SD) Abundance
L-2	Epistylidae	93.0	79.3	73.7 ± 67.0
	Unknown 1	84.5	16.8	14.2 ± 15.5
	Acinetidae	36.6	16.0	5.9 ± 15.2
	Lagenophryidae	36.6	9.8	3.6 ± 8.0
	Unknown 2	9.9	43.0	4.2 ± 13.5
	Stentoridae	0.0	0.0	0.0 ± 0.0
M-45	Epistylidae	100.0	104.3	104.3 ± 54.6
	Unknown 1	90.5	12.3	11.1 ± 8.0
	Acinetidae	38.1	14.8	5.6 ± 10.4
	Lagenophryidae	16.7	13.3	2.2 ± 6.6
	Unknown 2	2.4	12.5	0.3 ± 1.9
	Stentoridae	2.4	12.0	0.3 ± 1.9
X-2	Epistylidae	100.0	73.1	73.1 ± 46.1
	Unknown 1	83.3	12.2	10.2 ± 11.9
	Acinetidae	51.7	16.7	8.6 ± 12.7
	Lagenophryidae	16.7	13.9	2.3 ± 10.0
	Unknown 2	0.0	0.0	0.0 ± 0.0
	Stentoridae	0.0	0.0	0.0 ± 0.0

epibiont may have been introduced into the Great Lakes along with *Dreissena*. A variety of protists have been found associated with *Dreissena* (MOLLOY et al. 2001), and in at least three examples a protist was introduced into the Great Lakes via an exotic host species (GRIGOROVICH et al. 2001). Epibionts can have a negative impact on crustaceans by affecting movement, feeding, respiration, and reproduction (MORADO & SMALL 1995). In particular, epibionts attached to gills can cause metabolic changes affecting long-term survival of the host organism, make the host species more susceptible to other stresses (SCHUWERACK et al. 2001), or create lesions at points of attachment leading to enhanced microbial colonization (TURNER et al. 1979).

Despite contrasting trends in *Diporeia* densities at the three sites examined, the quantity, kind and distribution pattern of epibionts found attached to *Diporeia* at the sites were generally similar. The only observed difference between the sites was the greater number of attached Epistylidae found during the fall at sites where populations had already declined prior to or during our study (e.g. M-45 and L-2 respectively). If epibionts were the direct cause of population declines, then greater numbers would have been expected at these two sites over the entire sampling period when compared to X-2. Reasons for greater numbers of Epistylidae only during fall are not clear. WEISSMAN et al. (1993) found that epibionts might selectively colonize physiologically stressed animals. Therefore, increased epibiont loads at these two sites (M-45 and L-2) may have been a result of the animals being stressed by other factors, leading to behavioral traits conducive to epibiont attachment, such as decreased grooming or burrowing activity. Food limitation caused by the filtering activity of *Dreissena* is likely a major cause of physiological stress and possible mortality in *Diporeia* (NALEPA et al. 2005). *Dreissena* was abundant at M-45 and L-

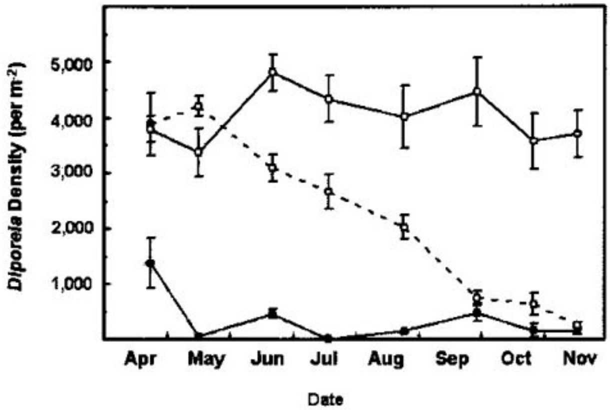

Fig. 2. Mean (± SE) density (per m^{-2}) of *Diporeia* at three sampling sites in Lake Michigan on each sampling date in 2002.
M-45 = ●—●; L-2 = ○ - - ○;
X-2 = ○—○

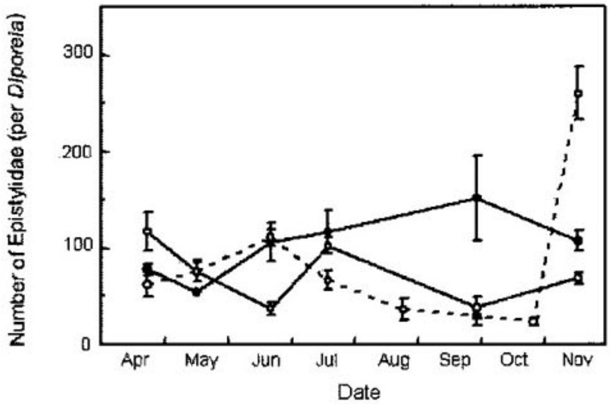

Fig. 3. Mean (± SE) number of attached Epistylidae per *Diporeia* at three sampling sites in Lake Michigan on each sampling date in 2002.
M-45 = ●—●; L-2 = ○ - - ○;
X-2 = ○—○

2, but was not present at X-2 (NALEPA unpubl. data). The observed fall peak in epibionts is consistent with seasonal trends found by others (GRIGOROVICH et al. 2001).

Several studies have documented the quantity and kind of epibionts attached to crustaceans in the Great Lakes. EVANS et al. (1979) determined the seasonal prevalence of the suctorian *Tokophrya quadripartita* on the calanoid copepod *Limnocalanus macrurus* in Lake Michigan, and GRIGOROVICH et al. (2001) documented the suctorian *Acineta nitocrae* on several species of harpacticoid copepods in Lake Erie and the Detroit River. Since our main intent was to document the total epibiont load relative to *Diporeia* density trends, our identifications of specific taxa are yet preliminary. However, the suctorian species found on *Diporeia* does not appear to be either *T. quadripartita* or *A. nitocrae*, but likely *Acineta gammari* (I.V. DOVGAL, pers. comm.).

To our knowledge this is the first study to document the total epibiont load on a benthic macrocrustacean in the Great Lakes. Even for other water bodies, studies of epibiont loads on macrocrustaceans are not common. Perhaps the most complete survey of attached epibionts was conducted on the isopod *Asellus aquaticus* from several chalk streams in England (COOK et al. 1998). A total of 13 genera comprising 16 protozoan taxa were identified on the 60 *Asellus* examined, and the mean number of attached epibionts per individual was 190. Using comparable methods (SEM), we found six different taxa (preliminary) on the 173 *Diporeia* examined, and the mean number per individual was 104. Peritrichs dominated the epibiont fauna in both studies; 89% on *Asellus* and 80% on *Di-*

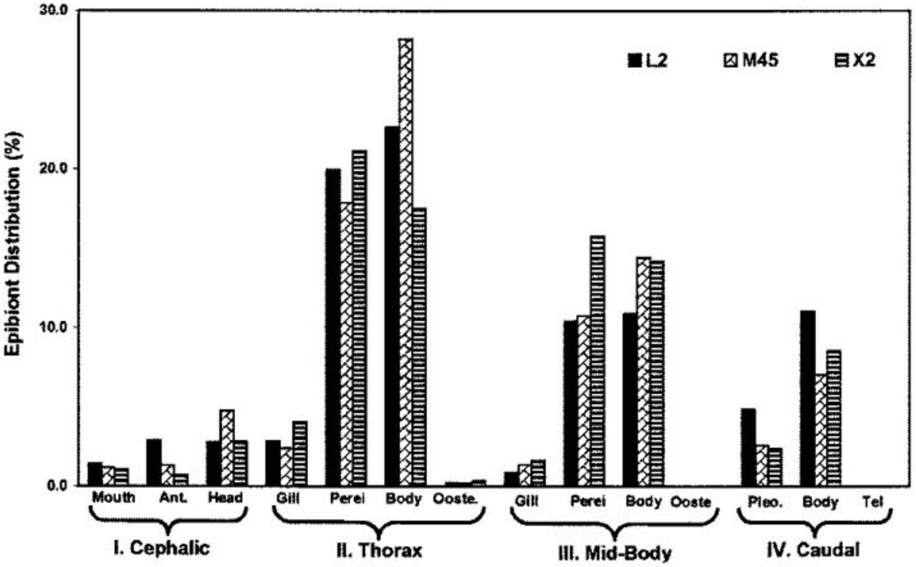

Fig. 4. Distribution of epibionts found attached to *Diporeia* at the three sampling sites. Values given as percentage of the total in 2002. See text for explanation of body regions.

poreia. Considering wide differences in environmental conditions in the two study areas, that is, the shallow, warm waters of chalk streams vs. the continually cold waters below the thermocline in Lake Michigan, the epibiont community found on *Asellus* and *Diporeia* were remarkably comparable.

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